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CLINICAL CASE SEMINAR

Hereditary Leiomyomatosis Associated with Bilateral, Massive, Macronodular Adrenocortical Disease and Atypical Cushing Syndrome: A Clinical and Molecular Genetic Investigation

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Hereditary leiomyomatosis and renal cell cancer (HLRCC) is an autosomal dominant disorder caused by mutations in the fumarate hydratase (*FH*) gene on chromosome 1q42.3–43. Massive macronodular adrenocortical disease (MMAD) is a heterogeneous condition associated with Cushing syndrome (CS) and bilateral hyperplasia of the adrenal glands. In MMAD, cortisol secretion is often mediated by ectopic, adrenocortical expression of receptors for a variety of substances; however, to date, no consistent genetic defects have been identified. In a patient with HLRCC caused by a germline-inactivating FH mutation, we diagnosed atypical (subclinical) CS due to bilateral, ACTH-independent adrenocortical hyperplasia. A clinical protocol for the detection of ectopic expression of various hormone receptors was employed. Histology was consistent with MMAD. The tumor tissue harbored the germline *FH* mutation and demonstrated allelic losses of the 1q42.3–43 *FH* locus. We then searched the National Institutes of Health (NIH) databases of patients with MMAD or HLRCC and found at least three other cases with MMAD that had a history of tumors that could be part of HLRCC; among patients with HLRCC, there were several with some adrenal nodularity noted on computed tomography but none with imaging findings consistent with MMAD. From two of the three MMAD patients, adrenocortical tumor DNA was available and sequenced for coding *FH* mutations; there were none. We conclude that in a patient with HLRCC, adrenal hyperplasia and CS were due to MMAD. The latter was likely due to the *FH* germline mutation because in tumor cells, only the mutant allele was retained. However, other patients with MMAD and HLRCC, or HLRCC patients with adrenal imaging findings consistent with MMAD, or MMAD patients with somatic FH mutations were not found among the NIH series. Although a fortuitous association cannot be excluded, HLRCC may be added to the short list of monogenic disorders that have been reported to be associated with the development of adrenal tumors; *FH* may be considered a candidate gene for MMAD. (***J Clin Endocrinol Metab* 90: 3773–3779, 2005**)

HEREDITARY LEIOMYOMATOSIS AND renal cell cancer (HLRCC) is an autosomal dominant disorder first described in 2001 by Launonen *et al.* (1). HLRCC is manifested by smooth muscle tumors of the skin (cutaneous leiomyomas), uterus (leiomyomas or leiomyosarcoma), and/or papillary renal carcinoma. Recently, germline mutations in the fumarate hydratase (*FH*) gene (on chromosome 1q42.3–43) have been found to be responsible for this disorder; several different mutations have been described in populations of mostly European and North American descent (2–4). FH is an enzyme that catalyzes the conversion of fumarate to malate in the tricarboxylic acid cycle; in HLRCC, the gene appears to act as a tumor suppressor because all patients are heterozygote carriers and, at the tumor level, there is loss of the normal allele. Supporting this notion is the fact that most mutations lead to nonsense mRNA (splicing, frameshift, and other changes), which leads to complete inactivation of FH activity at the tumor cell level.

In Launonen's original description of two families with HLRCC, two of 19 subjects had other tumors in addition to the skin, uterine, and renal cell tumors; one individual had breast cancer, and another had a tumor with an unknown primary site (1). Other tumors, including prostate and breast carcinomas, have also been described in mutation-positive individuals of HLRCC families (1, 5). On the other hand, somatic mutations of the *FH* gene appear to be infrequent. Recently, Lehtonen *et al.* (6) examined a series of 299 non-

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Abbreviations: CS, Cushing syndrome; CT, computed tomography; FH, fumarate hydratase; FISH, fluorescent *in situ* hybridization; HIF-1α, hypoxia-inducible factor type 1-α; HLRCC, hereditary leiomyomatosis and renal cell cancer; MAS, McCune-Albright syndrome; MMAD, massive macronodular adrenocortical disease; 17OHS, 17-hydroxysteroid; UFC, urinary free cortisol.

HLRCC-related, sporadic malignancies and found no pathogenic FH mutations. These tumors included colorectal, breast, lung, ovarian, testicular, thyroid, and head and neck cancers as well as pheochromocytomas, glioblastomas, and melanomas. Other studies examining sporadic tumors described in patients with HLRCC either did not find FH mutations (7) or found very low incidence of such genetic changes (8). For example, one study detected somatic FH mutations in a uterine leiomyosarcoma, a soft-tissue sarcoma, and a cutaneous leiomyoma (8); interestingly, two of the three mutations ended up being germline mutations (8) from patients that had previously undiagnosed HLRCC. Adrenocortical tumors have not been investigated for FH mutations.

We recently encountered a patient, who was known to belong to a large family with HLRCC caused by a germline FH mutation (3). She was found to have subclinical Cushing syndrome (CS) caused by massive macronodular adrenocortical disease (MMAD); her adrenal tumors showed molecular involvement of the FH locus. We also searched our databases for other patients with either MMAD and other tumors or HLRCC patients with adrenal tumors.

Subjects and Methods

Subjects and clinical protocols

All patients were studied at the NIH Warren Magnuson Clinical Center under institutional review board-approved protocols and after obtaining proper consent. The patients with MMAD, in particular, underwent a series of clinical studies under National Institute of Child Health and Human Development protocol 00-CH-0160. The following studies were obtained for the documentation and etiologic investigation of hypercortisolism: 1) an 0800 h plasma ACTH level followed by ovine CRH stimulation; 2) diurnal plasma cortisol variation, as previously described (9); 3) magnetic resonance imaging of the pituitary gland and computed tomography (CT) scan of the adrenal glands, as previously described (10); 4) a 6-d Liddle's test, as previously described (11): after 3 d of baseline urinary steroid excretion measurement, low-dose dexamethasone (0.5 mg/dose by mouth every 6 h) was given for 2 d, followed by high-dose dexamethasone (2 mg/dose every 6 h) for the last 2 d of the test; 24-h urine steroid excretion was measured daily; urinary free cortisol (UFC) was expressed per square meter of body surface area (micrograms per square meter per 24-h period), and 17-hydroxycorticosteroid excretion was expressed per grams of creatinine excreted in 24 h (milligrams per gram creatinine per 24-h period); and, finally, 5) testing for a variety of ectopic hormone receptor expression, as described elsewhere (12). Plasma ACTH and cortisol and UFC and 17-hydroxysteroid (17OHS) were measured, as previously described (9, 11). Characteristics for all assays (inter- and intraassay variations) have been previously published (9, 11).

Case report (case no. 1)

A 65-yr-old woman with characteristic skin findings of HLRCC (Fig. 1) presented with weight gain, hypertension, and hyperlipidemia. In the course of screening for tumors related to her genetic condition, bilateral adrenal enlargement was noted on CT (Fig. 2, *arrows*). Her past medical history was significant for a foot fracture several years before her evaluation at the NIH, a benign parotid gland tumor that had been excised elsewhere, and the most recent diagnosis of HLRCC. Her manifestations of the latter included diffuse coalescing painful cutaneous leiomyomas, uterine leiomyomas (for which she had undergone total hysteromyomectomy), but no renal tumors. Her medications included dibenzaline, nadolol, lipitor, and aspirin. She had no family history of endocrinopathies. Physical examination revealed a hypertensive (160/70), obese (with a body mass index of 36.6 kg/m^2) woman with diffuse cutaneous leiomyomata (Fig. 1). There were no striae, and only a suggestion of webbed neck was present.

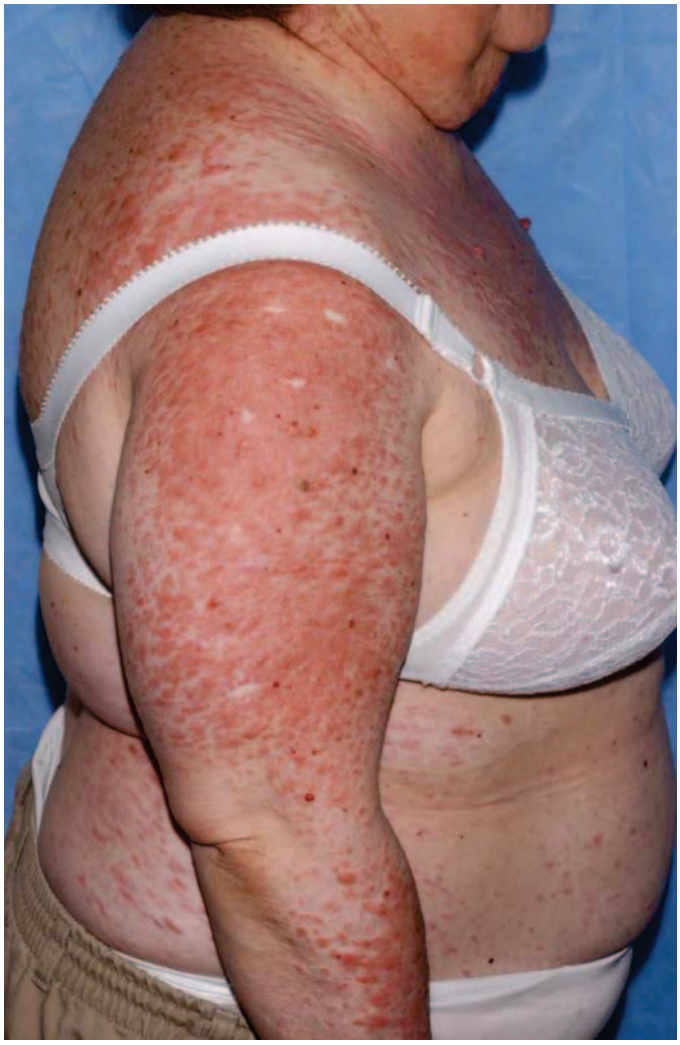

FIG. 1. Characteristic cutaneous leiomyomata covered the upper extremities of the patient.

Hormonal evaluation was significant for 24-h UFC, corrected for body surface area, of 77.8 μg/24 h (normal, 8–77 μg/d) and 17OHS, corrected for urinary creatinine, on a subsequent visit of 5.7 mg/24 h (normal, 2–6 mg/24 h). Urine and plasma catecholamines and serum

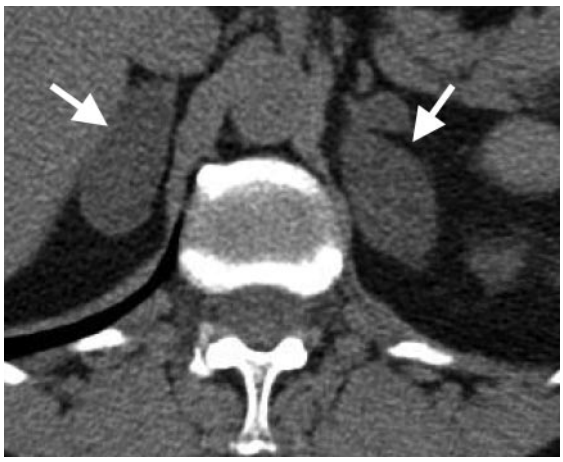

FIG. 2. Bilateral adrenal masses on CT (*arrows*).

potassium were normal. Fasting glucose was 122 mg/dl and 2-h oral glucose tolerance test blood glucose level was 179 mg/dl. Her serum ACTH values were low, ranging from undetectable to 17.4 pg/ml (normal, 9–52 pg/ml). Imaging studies showed the bilateral adrenal enlargement with heterogeneous enhancement after contrast CT. Several low-density nodules were seen; the larger, on the right side, measured 4.2 × 1.4 cm, and, on the left, 3.8 × 2.3 cm. Bone mineral density by dual x-ray absorptiometry revealed osteopenia in the femoral neck (T score, −2.2; Z score, −0.7) and osteoporosis in the lateral spine (L2–L4 T score, −3.4; Z score, −0.4). Diurnal cortisol measurements revealed 0730 and 0800 h levels of 25.8 and 22.1 μg/dl, respectively, and 2330 h and midnight values of 4.6 and 6.9 μg/dl, respectively. The ovine CRH-stimulation test showed an increase in both ACTH and cortisol values by 35 and 20%, respectively. A Liddle's test, however, demonstrated lack of UFC and 17OHS suppression (Table 1). Evaluation for the presence of abnormal cortisol responses included meal, posture, vasopressin, GnRH, glucagon, and GHRH tests, according to a protocol that has been published elsewhere (12); the posture and vasopressin tests showed a 100 and 54% increase in cortisol levels, respectively, compared with baseline (data not shown).

The patient underwent laparoscopic bilateral adrenalectomy without complications. She recovered and was discharged on hydrocortisone (20 mg in the morning and 10 mg at night) and florinef (100 μg/d). At 12 months postoperatively, the patient had lost 16 pounds, had better control of her blood pressure and serum glucose, and reported more energy compared with before the surgery. Her dose of hydrocortisone was reduced to a total of 25 mg/d. Interestingly, however, she complained of more discomfort from her leiomyomatosis compared with the preoperative period; skin pain has been treated with analgesics with some relief.

Search of other patients with MMAD and/or HLRCC

Records of 15 patients with MMAD studied at the NIH over the last 4 yr and those with HLRCC (from Ref. 3) were reviewed retrospectively for other manifestations that could constitute part of HLRCC or imaging findings consistent with MMAD, respectively. Three patients with MMAD were identified that could have had a multiple tumor syndrome consistent with HLRCC; from two of them, tumor DNA was extracted and sequenced for FH mutations (see below).

Tissue analysis

Tissue for genetic analysis was obtained at the time of surgery, frozen at −70 C, and stored for later use. For light microscopy and immunocytochemistry, tissue was paraffin-embedded; sections were then stained with hematoxylin and eosin and synaptophysin, as previously described (13–15). For electron microscopy, tissue was obtained at the time of surgery and processed as previously described (14, 15). Preparations of samples obtained at surgery from both adrenal glands and surrounding normal fibrous and fat tissue were processed for genetic analyses (see below).

DNA analysis

DNA was extracted from peripheral lymphocytes by standard methods (13, 14). Tumor DNA was extracted from frozen tissue in a 0.7-ml solution of 50 mM Tris (pH 8.0), 100 mM EDTA, 100 mM NaCl, 1% sodium dodecyl sulfate, and 0.5 mg/ml proteinase K. Samples were subsequently extracted four times in phenol/chloroform, precipitated with ethanol, and resuspended in 1× TE (50 mM Tris-HCl, 1 mM EDTA, pH 8.0).

TABLE 1. Liddle's test results

	UFC ($\mu g/24\ h \cdot m^2$)	17-OHS (mg/g creat·24 h)
d 1 (baseline)	56.4	6.2
d 2 (baseline)	53.9	5.9
d 3 (low-dose dex)	35.6	4.3
d 4 (low-dose dex)	19.2	2.9
d 5 (high-dose dex)	32.2	4.3
d 6 (high-dose dex)	35.4	5

dex, Dexamethasone.

Sequencing of the coding region of the *FH* gene was obtained following a protocol that we have described elsewhere (GeneDx, Rockville, MD) (3). Sequencing of the coding sequence of the *PRKAR1A* and *GNAS* genes was also obtained as we have described elsewhere (13, 16). Sequencing analysis of tissue-derived DNA was obtained as described elsewhere (13).

Fluorescent *in situ* hybridization (FISH) using bacterial artificial chromosomes containing control loci (such as one on chromosome 2p), the *FH* locus, and other chromosome 1q loci (such as one containing the *SDHC* gene on 1q21 and another containing the *PAP7* gene on 1q32) and the *PRKAR1A* (on 17q22–24) locus was performed as previously described (13). The bacterial artificial chromosomes were grown, and DNAs were extracted from them as we have described elsewhere (16); they were labeled with digoxigenin-11-deoxyuridine 5-triphosphate (Roche Molecular Biochemicals, Indianapolis, IN) by nick-translation and hybridized to touch-preparations of the tumor. After hybridization, cells were counterstained with 4′,6′-diamidino-2-phenylindol-dihydrochloride. Hybridization signals were analyzed with the use of a Leica epifluorescence microscope (Leica Corp.), and fluorescence images were automatically captured on a Photometrics cooled-CCD camera (Photometrics, Tucson, AZ) using IP Lab Image software (Scanalytics, Inc., Fairfax, VA). Two hundred interphases with strong hybridization signals were scored. Presence of more than 25% cells with only one signal was interpreted as indicative of an allelic deletion. SpectrumGreen or SpectrumOrange α-satellite probes (Vysis, Inc., Donners Grove, IL) were used for chromosome identification.

Results

Clinical outcome of case no. 1

The patient with HLRCC and MMAD did well postoperatively; at the 12-month check up, she had lost more than 10% of her preoperative weight. Blood pressure and glucose control improved dramatically (data not shown). Interestingly, however, her skin leiomyomata were more symptomatic: pruritus, erythema, and pain became more frequent in the first 6 months postoperatively, with some improvement thereafter.

Search for other patients with MMAD and/or HLRCC

Records of 15 patients with MMAD studied at the NIH over the last 4 yr and those with HLRCC (from Ref. 3) were reviewed retrospectively for other manifestations (that could be part of HLRCC) or adrenal gland imaging findings, respectively. Three patients with MMAD and other tumors were identified; all three were females in their mid-40s or 50s with history of uterine lesions and hysterectomies. One had, in addition, history of benign head and neck tumors. None had history of renal tumors or skin manifestations consistent with leiomyomatosis. Among the HLRCC patients that were seen at the NIH and reported in Ref. 3 (including the siblings of case no. 1), there were none with imaging findings consistent with MMAD, although several cases with adrenal nodularity were noted (data not shown). From two of the three patients with MMAD and history of other lesions, tumor DNA was extracted and sequenced for *FH* mutations (see below).

Histology (case no. 1)

The right adrenal gland weighed 48 g and measured 7 × 4.5 × 3 cm; the left adrenal gland weighed 57.4 g and measured 7 × 4.5 × 3 cm. Diffuse cortical hyperplasia, as had

been suggested by the imaging studies (Fig. 2, *arrows*), was found. The normal cortical architecture in both adrenals was replaced by a predominantly diffuse and vaguely nodular proliferation of clear cortical cells with ample cytoplasm. Clear cells of the usual size were also present, but compact cells of small or conventional size were rarely seen. A small rim of zona glomerulosa cells was present at the periphery. In addition, there was increased microvessel density in both adrenals and interspersed cavernous hemangiomas in the left adrenal (which are not regularly seen in MMAD or other adrenal tumors) (Fig. 3, A–C). Ultrastructurally, the cytoplasm of almost all cells was filled with an excessive amount of large lipid vacuoles. In addition, increased numbers of round mitochondria, stacks of RER, and dense lysosomal type bodies were seen (Fig. 3D).

Molecular genetic studies

Case no. 1 is a member of a family with HLRCC that was included in a publication of the NIH series of patients with this genetic condition (3). For the purposes of the present study, her blood, tumor, and normal tissue DNA were sequenced; this analysis revealed a germline mutation in the *FH* gene consisting of a 7-bp deletion at nucleotides 782–788 (c.781del7), leading to a premature stop codon at position 261 of the protein (P261X), consistent with the reported data. The patient was heterozygous for this mutation in peripheral blood lymphocytes, but only one copy of the *FH* gene, the mutant allele, was retained by the adrenal tissue, suggesting loss of the normal allele or loss of heterozygosity (data not shown).

FISH analysis confirmed the allelic loss of the *FH* gene in the excised adrenocortical tissue (Fig. 4, A–C). Control probes from other chromosomes showed the expected two signals (the one from chromosome 2p is shown in Fig. 4D). Probes from other chromosome 1q loci showed a progressively increasing incidence of allelic losses approaching the 1q42.3–43 *FH* locus; for example, the probe containing a gene (*SDHC*) on 1q21 showed losses in 17% of tumor cells, whereas one from the 1q32 region (containing the *PAP7* gene), which is more proximal to the 1q42.3–43 *FH* locus, showed losses in 30% of tumor cells. The incidence of allelic losses in the patient's tumor cells for probes from the 1q chromosomal region peaked around the 1q42.3–43 *FH* locus at approximately 50%. A representative experiment is shown in Fig. 5; in this image, only one (indicated by the *arrow*) of the seven cells from the patient's tumor showed allelic losses for the *SDHC* gene, compared with 50% of the cells shown in Fig. 4C.

As mentioned above, from two of the three patients with MMAD and history of other lesions, samples were available, and tumor DNA was extracted. There were no *FH* coding sequence mutations in either. For all three patients, including that of case no. 1, tumor DNA was studied for *PRKAR1A* or

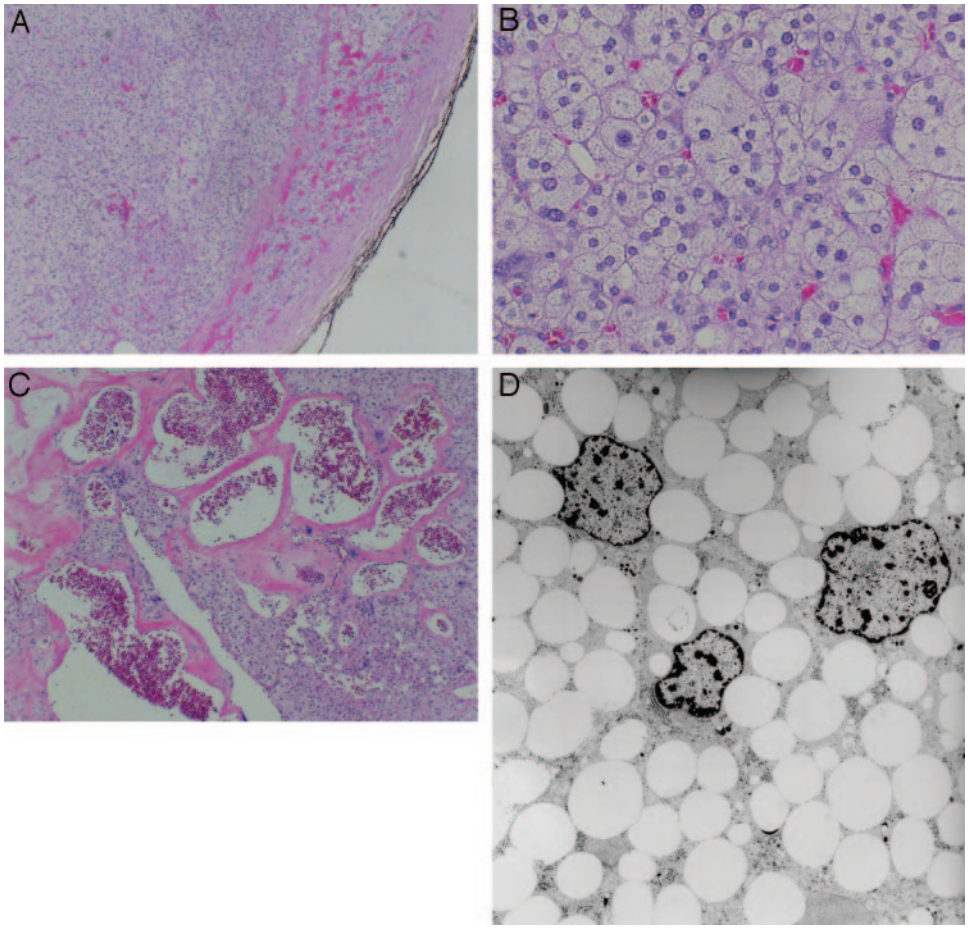

FIG. 3. A, A cortical hyperplastic nodule, clearly delineated from the normal cortical tissue (×40). B, A higher magnification of cortical hyperplasia (×100). C, Multiple cavernous hemangiomata were also seen on histological examination (×100). D, Electron microscopy studies showed lipid accumulation, round mitochondria, and other signs of adrenocortical hyperplasia.

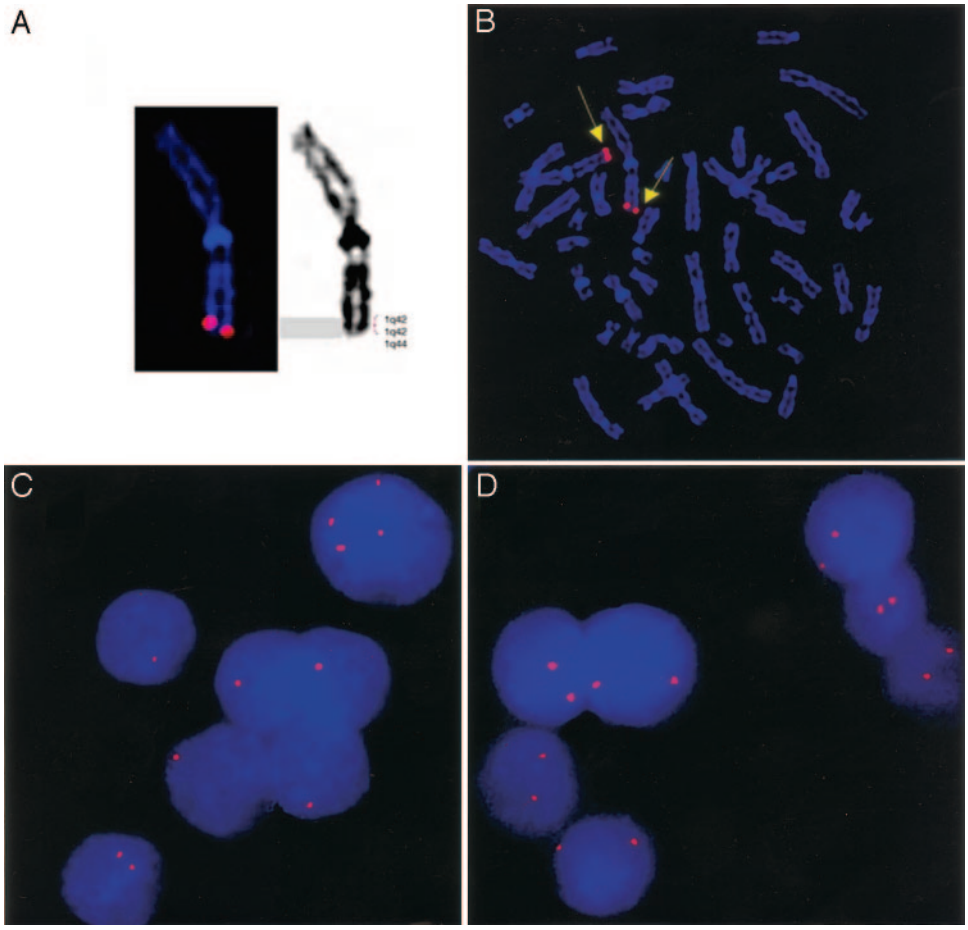

FIG. 4. Mapping of the *FH* gene and deletion of the 1q42.3–43 region in the patient's tumor tissue; the *red signal* is from the probe containing the *FH* gene. A and B, On a control hybridization of a metaphase chromosome preparation from normal cells, the *FH* gene probe localized to the 1q42.3–43 region; the *arrows* show the position of the *FH* gene at a 4′,6′-diamidino-2-phenylindol-dihydrochloride-counterstained chromosome 1. C, Interphase FISH with the same probe to adrenocortical tumor cells prepared from the patient showed one signal in more than 50% of the cells (*red*). D, A control FISH experiment with a similarly prepared probe from chromosome 2 (also *in red*) on the same tumor cells showed the expected two signals.

GNAS somatic mutations; none was identified (data not shown).

Discussion

Adrenocortical tumors may be found in the adrenal glands of patients with congenital adrenocortical hyperplasia (17), multiple endocrine neoplasia type 1 (18–20), McCune-Albright syndrome (MAS) (21–23), familial polyposis coli (24, 25), and Carney complex (13, 16, 26). *GNAS* or *PRKAR1A* mutations, the MAS and Carney complex genes, respectively, only rarely occur somatically in sporadic adrenocortical tumors (13, 27, 28) and do not appear to be frequently mutated in MMAD or ACTH-independent macronodular adrenocortical hyperplasia. MMAD is a rare disorder that is associated with classic, as well as atypical, forms of CS (29, 30). It was first described in 1964 by Kirschner *et al.* (29) in a 40-yr-old woman who presented with long-standing, ACTH-independent CS. The patient underwent bilateral adrenalectomy; her adrenal glands had multiple nodules and a combined weight of 94 g. Since 1964, this patient's disease has been seen in tens of patients and described under different names, including ACTH-independent macronodular adrenocortical hyperplasia, MMAD, autonomous macronodular adrenal hyperplasia, ACTH-independent massive bilateral adrenal disease, and giant or huge macronodular adrenal disease. MMAD seems to have a bimodal age distribution; few patients present during the first year of life (this form of the disease may be associated with MAS), whereas most present in the fifth decade of life, just like the patient of this report.

MMAD rarely occurs in families, suggesting that, for some patients, an autosomal dominant predisposition exists for the development of bilateral, large adrenocortical tumors (31). MMAD has also been associated with somatic mutations of the *GNAS* gene in infants with MAS and some older patients with macronodular adrenocortical enlargement (22, 23, 27, 31). However, neither inheritance nor *GNAS* genetic defects appear to explain the vast majority of MMAD cases. In them, ectopic neuroendocrine hormone receptor expression has been the most common and clinically useful feature (31), a feature that is also increasingly found in sporadic, non-MMAD-related, adrenal adenomas (32): cortisol secretion in MMAD appears to be regulated by aberrant (adrenocortical) expression of receptors for various hormones that are not present physiologically in cortical tissue, such as those for gastric inhibitory polypeptide, vasopressin, α-adrenergic agonists, serotonin, and LH/human chorionic gonadotropin (31). The presence of these receptors suggests a link to the cAMP-dependent protein kinase signaling; however, no *PRKAR1A* mutations have been identified to date, and other components of this pathway may be functionally altered but are not somatically mutated (33). Part of the problem in the genetic characterization of MMAD is its apparent molecular heterogeneity that follows its diverse clinical features (33).

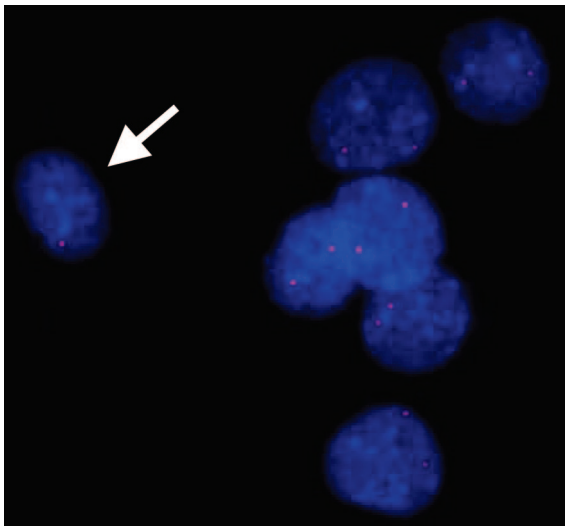

FIG. 5. A probe (*red signal*) containing the *SDHC* gene (which maps to the 1q21 region centromeric to the *FH* locus) shows mostly two signals in the patient's tumor cells. Only one of the seven cells in this picture has lost one copy of the *SDHC* gene (*arrow*); the incidence of allelic losses in the patient's tumor cells increased along the length of chromosome 1q and peaked around the FH locus on 1q42.3–43 (data not shown).

Studies like the present one, based on rare patients, could therefore be very useful because they may provide a previously unsuspected link to an existing molecular pathway.

Could FH be linked causatively to adrenocortical tumorigenesis? The enzyme participates in the Krebs cycle and is expressed in all tissues, including the adrenal cortex (34). Its involvement in causing a variety of tumors appears to follow that of another mitochondrial multimeric protein that has recently been found to function as a tumor suppressor, succinate dehydrogenase. Succinate accumulates as a result of succinate dehydrogenase/FH inhibition; excess succinate inhibits hypoxia-inducible factor type 1α (HIF-1α) hydroxylases, which leads to stabilization and activation of HIF-1α (35). HIF-1α is expressed in adrenal tissue and regulated by glucocorticoids (36). Adaptation to hypoxia is affected by glucocorticoids; hypoxia-dependent signals and glucocorticoid-regulated genes are coordinated processes (36). A molecule, called endocrine-gland-derived vascular endothelial growth factor, appears to induce endothelial cell proliferation in endocrine tissues; endocrine-gland-derived vascular endothelial growth factor possesses a HIF-1α binding site, and its expression is induced by hypoxia (37). We may speculate that the unusual finding of interspersed cavernous hemangiomata in our patient (Fig. 3C) is the result of overexpression of such an angiogenic factor due to HIF-1α induction caused by the FH mutation. It is conceivable that adrenocortical cell proliferation followed these phenomena, as a consequence of overexpression of these factors, as has been postulated for other tumors caused by *FH* mutations (38).

Finally, we should comment on the treatment of the described patient (case no. 1). Like a number of patients with MMAD and other adrenal tumors discovered in the course of imaging studies for a variety of diseases or unrelated symptoms, our patient was found to have subclinical or atypical CS only after a CT was obtained for her underlying genetic disorder; a complete biochemical investigation was then initiated. However, not only did she not have overt CS clinically, but her ACTH levels were not suppressed, and her UFCs were minimally elevated at baseline. A number of patients like her have been described recently (30, 39–42). According to a recent NIH conference and other investigators (30, 39, 43), the treatment of choice for patients with subclinical CS caused by incidentally discovered adrenal tumors is surgical (44). Medical adrenalectomy is not recommended because of the associated side effects, inefficient ablation of cortisol secretion, and need for frequent medical follow-up visits (43). Laparoscopy has decreased the morbidity, length of hospitalization, and long-term side effects of adrenalectomy in these patients (39). Improvement of a number of indices, from bone density to blood pressure and glucose intolerance, has been recorded in patients with incidentally discovered adrenal masses and subclinical CS who underwent adrenalectomy (30, 40–42).

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References

1. **Launonen V, Vierimaa O, Kiuru M, Isola J, Roth S, Pukkala E, Sistonen P, Herva R, Aaltonen LA** 2001 Inherited susceptibility to uterine leiomyomas and renal cell cancer. Proc Natl Acad Sci USA 98:3387–3392
2. **Linehan WM, Walther MM, Zbar B** 2003 The genetic basis of cancer of the kidney. J Urol 170:2163–2172
3. **Toro JR, Nickerson ML, Wei MH, Warren MB, Glenn GM, Turner ML, Stewart L, Duray P, Tourre O, Sharma N, Choyke P, Stratton P, Merino M, Walther MM, Linehan WM, Schmidt LS, Zbar B** 2003 Mutations in the fumarate hydratase gene cause hereditary leiomyomatosis and renal cell cancer in families in North America. Am J Hum Genet 73:95–106
4. **Alam NA, Bevan S, Churchman M, Barclay E, Barker K, Jaeger EE, Nelson HM, Healy E, Pembroke AC, Friedmann PS, Dalziel K, Calonje E, Anderson J, August PJ, Davies MG, Felix R, Munro CS, Murdoch M, Rendall J, Kennedy S, Leigh IM, Kelsell DP, Tomlinson IP, Houlston RS** 2001 Localization of a gene (MCUL1) for multiple cutaneous leiomyomata and uterine fibroids to chromosome 1q42.3-q43. Am J Hum Genet 68:1264–1269
5. **Kiuru M, Launonen V, Hietala M, Aittomaki K, Vierimaa O, Salovaara R, Arola J, Pukkala E, Sistonen P, Herva R, Aaltonen LA** 2001 Familial cutaneous leiomyomatosis is a two-hit condition associated with renal cell cancer of characteristic histopathology. Am J Pathol 159:825–829
6. **Lehtonen R, Kiuru M, Vanharanta S, Sjoberg J, Aaltonen LM, Aittomaki K, Arola J, Butzow R, Eng C, Husgafvel-Pursiainen K, Isola J, Jarvinen H, Koivisto P, Mecklin JP, Peltomaki P, Salovaara R, Wasenius VM, Karhu A, Launonen V, Nupponen NN, Aaltonen LA** 2004 Biallelic inactivation of fumarate hydratase (FH) occurs in nonsyndromic uterine leiomyomas but is rare in other tumors. Am J Pathol 164:17–22
7. **Barker KT, Bevan S, Wang R, Lu YJ, Flanagan AM, Bridge JA, Fisher C, Finlayson CJ, Shipley J, Houlston RS** 2002 Low frequency of somatic mutations in the FH/multiple cutaneous leiomyomatosis gene in sporadic leiomyosarcomas and uterine leiomyomas. Br J Cancer 87:446–448
8. **Kiuru M, Lehtonen R, Arola J, Salovaara R, Jarvinen H, Aittomaki K, Sjoberg J, Visakorpi T, Knuutila S, Isola J, Delahunt B, Herva R, Launonen V, Karhu A, Aaltonen LA** 2002 Few FH mutations in sporadic counterparts of tumor types observed in hereditary leiomyomatosis and renal cell cancer families. Cancer Res 62:4554–4557
9. **Papanicolaou DA, Yanovski JA, Cutler Jr GB, Chrousos GP, Nieman LK** 1998 A single midnight serum cortisol measurement distinguishes Cushing's syndrome from pseudo-Cushing states. J Clin Endocrinol Metab 83:1163–1167

10. **Doppman JL, Chrousos GP, Papanicolaou DA, Stratakis CA, Alexander HR, Nieman LK** 2000 Adrenocorticotropin-independent macronodular adrenal hyperplasia: an uncommon cause of primary adrenal hypercortisolism. Radiology 216:797–802
11. **Stratakis CA, Sarlis N, Kirschner LS, Carney JA, Doppman JL, Nieman LK, Chrousos GP, Papanicolaou DA** 1999 Paradoxical response to dexamethasone in the diagnosis of primary pigmented nodular adrenocortical disease. Ann Intern Med 131:585–591
12. **Lacroix A, Ndiaye N, Tremblay J, Hamet P** 2001 Ectopic and abnormal hormone receptors in adrenal Cushing's syndrome. Endocr Rev 22:75–110
13. **Bertherat J, Groussin L, Sandrini F, Matyakhina L, Bei T, Stergiopoulos S, Papageorgiou T, Bourdeau I, Kirschner LS, Vincent-Dejean C, Perlemoine K, Gicquel C, Bertagna X, Stratakis CA** 2003 Molecular and functional analysis of PRKAR1A and its locus (17q22–24) in sporadic adrenocortical tumors: 17q losses, somatic mutations, and protein kinase A expression and activity. Cancer Res 63:5308–5319
14. **Gunther DF, Bourdeau I, Matyakhina L, Cassarino D, Kleiner DE, Griffin K, Courkoutsakis N, Abu-Asab M, Tsokos M, Keil M, Carney JA, Stratakis CA** 2004 Cyclical Cushing syndrome presenting in infancy: an early form of primary pigmented nodular adrenocortical disease, or a new entity? J Clin Endocrinol Metab 89:3173–3182
15. **Courcoutsakis NA, Patronas NJ, Cassarino D, Griffin K, Keil M, Ross JL, Carney JA, Stratakis CA** 2004 Hypodense nodularity on computed tomography: novel imaging and pathology of micronodular adrenocortical hyperplasia associated with myelolipomatous changes. J Clin Endocrinol Metab 89:3737–3738
16. **Kirschner LS, Carney JA, Pack SD, Taymans SE, Giatzakis C, Cho YS, Cho-Chung YS, Stratakis CA** 2000 Mutations of the gene encoding the protein kinase A type I-α regulatory subunit in patients with the Carney complex. Nat Genet 26:89–92
17. **Ravichandran R, Lafferty F, McGinniss MJ, Taylor HC** 1996 Congenital adrenal hyperplasia presenting as massive adrenal incidentalomas in the sixth decade of life: report of two patients with 21-hydroxylase deficiency. J Clin Endocrinol Metab 81:1776–1779
18. **Skogseid B, Larsson C, Lindgren PG, Kvanta E, Rastad J, Theodorsson E, Wide L, Wilander E, Oberg K** 1992 Clinical and genetic features of adrenocortical lesions in multiple endocrine neoplasia type 1. J Clin Endocrinol Metab 75:76–81
19. **Burgess JR, Harle RA, Tucker P, Parameswaran V, Davies P, Greenaway TM, Shepherd JJ** 1996 Adrenal lesions in a large kindred with multiple endocrine neoplasia type 1. Arch Surg 131:699–702
20. **Barzon L, Pasquali C, Grigoletto C, Pedrazzoli S, Boscaro M, Fallo F** 2001 Multiple endocrine neoplasia type 1 and adrenal lesions. J Urol 166:24–27
21. **Weinstein LS, Shenker A, Gejman PV, Merino MJ, Friedman E, Spiegel AM** 1991 Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. N Engl J Med 325:1688–1695
22. **Kirk JM, Brain CE, Carson DJ, Hyde JC, Grant DB** 1999 Cushing's syndrome caused by nodular adrenal hyperplasia in children with McCune-Albright syndrome. J Pediatr 134:789–792
23. **Mantovani G, Bondioni S, Lania AG, Corbetta S, de Sanctis L, Cappa M, Di Battista E, Chanson P, Beck-Peccoz P, Spada A** 2004 Parental origin of Gsα mutations in the McCune-Albright syndrome and in isolated endocrine tumors. J Clin Endocrinol Metab 89:3007–3009
24. **Kartheuser A, Walon C, West S, Breukel C, Detry R, Gribomont AC, Hamzehloei T, Hoang P, Maiter D, Pringot J, Rahier J, Khan PM, Curtis A, Burn J, Fodde R, Verellen-Dumoulin C** 1999 Familial adenomatous polyposis associated with multiple adrenal adenomas in a patient with a rare 3′ APC mutation. J Med Genet 36:65–67
25. **Blaker H, Sutter C, Kadmon M, Otto HF, Von Knebel-Doeberitz M, Gebert J, Helmke BM** 2004 Analysis of somatic APC mutations in rare extracolonic tumors of patients with familial adenomatous polyposis coli. Genes Chromosomes Cancer 41:93–98
26. **Stratakis CA** 2002 Mutations of the gene encoding the protein kinase A type I-α regulatory subunit (*PRKAR1A*) in patients with the "complex of spotty skin pigmentation, myxomas, endocrine overactivity, and schwannomas" (Carney complex). Ann NY Acad Sci 968:3–21
27. **Fragoso MC, Domenice S, Latronico AC, Martin RM, Pereira MA, Zerbini MC, Lucon AM, Mendonca BB** 2003 Cushing's syndrome secondary to adrenocorticotropin-independent macronodular adrenocortical hyperplasia due to activating mutations of *GNAS1* gene. J Clin Endocrinol Metab 88:2147–2151
28. **Stratakis CA** 2003 Genetics of adrenocortical tumors: gatekeepers, landscapers and conductors in symphony. Trends Endocrinol Metab 14:404–410
29. **Kirschner MA, Powell Jr RD, Lipsett MB** 1964 Cushing's syndrome: nodular cortical hyperplasia of adrenal glands with clinical and pathological features suggesting adrenocortical tumor. J Clin Endocrinol Metab 24:947–955
30. **Sippel RS, Chen H** 2004 Subclinical Cushing's syndrome in adrenal incidentalomas. Surg Clin North Am 84:875–885
31. **Bourdeau I, Stratakis CA** 2002 Cyclic AMP-dependent signaling aberrations in macronodular adrenal disease. Ann NY Acad Sci 968:240–255
32. **Groussin L, Perlemoine K, Contesse V, Lefebvre H, Tabarin A, Thieblot P, Schlienger JL, Luton JP, Bertagna X, Bertherat J** 2002 The ectopic expression of the gastric inhibitory peptide (GIP) receptor is frequent in ACTH-independent macronodular adrenal hyperplasia but rare in benign adrenal adenomas. J Clin Endocrinol Metab 87:1980–1985
33. **Bourdeau I, Antonini SR, Lacroix A, Kirschner LS, Matyakhina L, Lorang D, Libutti SK, Stratakis CA** 2004 Gene array analysis of macronodular adrenal hyperplasia confirms clinical heterogeneity and identifies several candidate genes as molecular mediators. Oncogene 23:1575–1585
34. **Nelson JS** 1973 The quantitative histochemistry of the sympathoadrenal system. II. Enzymes of the citric acid, pentose phosphate and glycogenolytic pathways. J Histochem Cytochem 21:483–487
35. **Selak MA, Armour SM, Mackenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Simon MC, Thompson CB, Gottlieb E** 2005 Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-α prolyl hydroxylase. Cancer Cell 7:77–85
36. **Kodama T, Shimizu N, Yoshikawa N, Makino Y, Ouchida R, Okamoto K, Hisada T, Nakamura H, Morimoto C, Tanaka H** 2003 Role of the glucocorticoid receptor for regulation of hypoxia-dependent gene expression. J Biol Chem 278:33384–33391
37. **LeCouter J, Kowalski J, Foster J, Hass P, Zhang Z, Dillard-Telm L, Frantz G, Rangell L, DeGuzman L, Keller GA, Peale F, Gurney A, Hillan KJ, Ferrara N** 2001 Identification of an angiogenic mitogen selective for endocrine gland endothelium. Nature 412:877–884
38. **McGrath JA** 2003 Biologic lessons from mutations in the Krebs cycle enzyme, fumarate hydratase. J Invest Dermatol 121:vii
39. **Thompson GB, Young Jr WF** 2003 Adrenal incidentaloma. Curr Opin Oncol 15:84–90
40. **Libe R, Dall'Asta C, Barbetta L, Baccarelli A, Beck-Peccoz P, Ambrosi B** 2002 Long-term follow-up study of patients with adrenal incidentalomas. Eur J Endocrinol 147:489–494
41. **Hadjidakis D, Tsagarakis S, Roboti C, Sfakianakis M, Iconomidou V, Raptis SA, Thalassinos N** 2003 Does subclinical hypercortisolism adversely affect the bone mineral density of patients with adrenal incidentalomas? Clin Endocrinol (Oxf) 58:72–77
42. **Bernini G, Moretti A, Iacconi P, Miccoli P, Nami R, Lucani B, Salvetti A** 2003 Anthropometric, haemodynamic, humoral and hormonal evaluation in patients with incidental adrenocortical adenomas before and after surgery. Eur J Endocrinol 148:213–219
43. **Grumbach MM, Biller BM, Braunstein GD, Campbell KK, Carney JA, Godley PA, Harris EL, Lee JK, Oertel YC, Posner MC, Schlechte JA, Wieand HS** 2003 NIH Consensus Conference: management of the clinically inapparent adrenal mass ("incidentaloma"). Ann Intern Med 138:424–429
44. **Dluhy RG, Maher MM, Wu CL** 2005 Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 7-2005. A 59-year-old woman with an incidentally discovered adrenal nodule. N Engl J Med 352:1025–1032

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